A Critical Role for YPK1 in Farnesol Mediated Modulation of Aureobasidin A Drug Susceptibility

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Short Report

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1 **A Critical role for *YPK1* in farnesol mediated modulation of Aureobasidin A drug**
2 **susceptibility**

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Abstracts

Candida albicans is a dimorphic fungus, have the ability to secrete a quorum sensing molecule farnesol in a cell density dependent manner which regulates its morphogenic shift from yeast to hyphae. The ability to switch between different forms is highly correlated with its ability to cause disease such as Candidiasis. The switching between different yeast to hyphae form leads to significant alteration in the plasma membrane sphingolipids. Complex sphingolipids are principal components of fungal plasma membranes; and their interaction with antifungal drug can play a crucial role in multidrug resistance. Therefore, we reasoned that the farnesol mediated modulation of the complex sphingolipid biosynthetic pathway might lead to the altered drugs susceptibility. Our studies demonstrate for the first time that exogenous addition of farnesol affects the sphingolipid biosynthesis and causes altered Aureobasidin A (AbA) drug susceptibility which mainly inhibits the complex sphingolipid biosynthesis pathway.

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Introduction

Candida albicans is an opportunistic pathogenic yeast that exist as a member of human gut flora. Usually it is a commensal organism, but it can become pathogenic in immunocompromised individuals under a variety of conditions and it causes severe life-threatening infection (Kim and Sudbery 2011; Martins et al. 2014). *C. albicans*, can grow as both, yeast and filamentous forms and the ability to switch between different morphogenic forms is highly correlated with its ability to cause disease (Hornby and Nickerson 2004). *C. albicans* have the ability to secrete the quorum sensing molecule farnesol which inhibits yeast-to-filamentous form conversion (Nickerson et al. 2006), resulting in mainly actively budding yeasts without any filamentous growth that could affect the drug susceptibility of this pathogen (Hornby et al. 2001). The extensive and long-term usage of antifungal drugs in recent years has caused rapid development of drug resistance, which leads to major threats to antifungal therapy (Tanwar et al. 2014; Zhai and Lin 2011; Prasad et al. 2002). Recently, it has been shown that the deletion of *FEN1* and *SUR4* gene involved in sphingolipid biosynthetic pathway in *Saccharomyces cerevisiae*, and its homolog in *C. albicans* sensitize them against polyene amphotericin B (AmB) (Sharma et al. 2014). Similarly, another study demonstrated that plasma membrane proteolipid 3 protein affects AmB resistance through the sphingolipid biosynthesis pathway (Bari et al. 2015). Moreover, disruption of the interaction between membrane ergosterols and sphingolipids or alteration in membrane lipid composition can modulate the antifungal drug susceptibilities of *C. albicans* (Mukhopadhyay et al. 2004; Loffler et al. 2000; Mukhopadhyay et al. 2002). In conclusion sphingolipid molecules emerged as potential effectors in modulation of antifungal drug susceptibility. Therefore, here we studied the impact of farnesol on sphingolipids biosynthesis and their involvement in the antifungal drug resistance of *C. albicans*, we used AbA drug which acts as an inhibitor of complex sphingolipids biosynthesis of *S. cerevisiae* (Sugimoto et al. 2004; Cerantola et al. 2009a).

Complex sphingolipids are important structural components of the plasma membrane and play an important role as signaling molecules (Dickson et al. 2006; Dickson 2008). Yeast cells can synthesize three type of complex sphingolipids containing inositol phosphates (IP) derived moiety namely Inositolphosphoryl-ceramide (IPC) Mannosyl-inositolphosphoryl-ceramides (MIPC) and Mannosyl-di(inositolphosphoryl)-ceramides (M(IP)₂C) (Culbertson et al. 1976). AbA is a potent and specific inhibitor of phosphatidylinositol: ceramide phosphoinositol transferase (Aur1p), which catalyzes the first step in complex sphingolipid synthesis (Cerantola et al. 2009b; Heidler and Radding 1995). Our study demonstrates that farnesol can suppress the inhibitory effect of AbA drugs in *S.cerevisiae* and *Candida* strains.

Material and methods

Strains, media and growth conditions

S. cerevisiae deletion mutants were obtained from the euro scarf. Fine chemicals and yeast synthetic complete (SC) medium components were obtained from Himedia. Stock solutions of AbA (2 mg/ml), were prepared in DMSO. Farnesol stock solutions (50 mM) were made in DMSO.

Growth assays by dilution spotting. For dilution spotting assays, the strains were grown overnight in SC medium, reinoculated in fresh medium to an Abs_{600nm} of 0.1 and grown for 6 h. The exponential phase cells were harvested, washed and resuspended in sterile water to an Abs_{600nm} of 1.0. Ten-fold serial dilutions were made in water and 5 µl of each dilution was spotted on desired plates. Plates were incubated for 2 days at 30° C before taking photographs.

Result and discussion

While investigating the apparent role of the sphingolipid biosynthesis pathway in antifungal drug resistance on solid media, we speculated that farnesol that accumulates in a cell-density dependent manner might be responsible for the increased resistance against antifungal drugs.

Therefore, the effect of farnesol on complex sphingolipid biosynthesis pathway inhibitor AbA (**Fig. 1a**) was checked using *S. cerevisiae* and *Candida* strains. Interestingly, we observed that the minimum inhibitory concentration of AbA drug in *S.cerevisiae* strain (FY4, BY4741), *Candida albicans* (SC5314, SN95) *Candida glabrata* (CG462), *Candida lusitania* (CL1 & CL6) and *Candida tropicalis* (CT3404), increases in presence of the 50 μ M farnesol by two to four-fold (**Fig. 1b**). This increase in resistance suggests that farnesol is one of the factors that influence AbA resistance. Since farnesol increases the resistance of all the tested strains of *S. cerevisiae* by about 2 to 4-folds, it appears that it acts at least partly through the sphingolipid biosynthesis pathway. Further, to identify the crucial gene(s) that are involved in farnesol mediated increase in AbA resistance; we screened selected deletion collections of *S.cerevisiae* that upon deletion modulates AbA resistance, particularly in the presence of farnesol. *S.cerevisiae* gene deletant defective in major regulators of the pleiotropic drug response (PDR deletants), ergosterol biosynthesis (ERG deletants), calcineurin pathway and sphingolipid biosynthesis regulators were screen against AbA with and without farnesol.

Using plate-based dilution spot assay, first we started to screen gene deletion mutants that are directly involved in sphingolipid biosynthesis regulation, because these regulators play crucial roles in sphingolipid biosynthesis and its homeostasis (**Fig. 2a**). Moreover, we have screened the deletion mutants of *S.cerevisiae* which are defective in pleiotropic drug response acting as drug efflux since these efflux proteins can also modulate our farnesol mediated AbA drug resistance phenotype (**Fig. 2b**). Interestingly, we have identified pleiotropic drug response 5 (*PDR5*) encoding plasma membrane ATP-binding cassette (ABC) transporter and Yeast Protein Kinase 1 (*YPK1*) gene, respectively, which in the presence of farnesol affect the AbA resistance substantially out of all the strain tested as compared to the parent strain. These data demonstrate that *PDR5* and *YPK1* are crucial for farnesol-mediated increased AbA resistance, while other gene(s) do not influence AbA resistance as much. Sphingolipids and ergosterol act

are major yeast plasma membrane component, and interaction between these molecules might play a crucial role in drug susceptibility (Mukhopadhyay et al. 2004), hence to rule the possible involvement of ergosterol in AbA drug susceptibility, we also analyze AbA susceptibility of the ergosterol biosynthetic pathway mutant in presence of farnesol, however we did not find correlation between ergosterol biosynthetic mutants and AbA drug resistance (**Fig. 3a**). Sphingolipid biosynthesis is modulated by calcineurin signaling pathways (Gururaj et al. 2013). Calcineurin is a heterodimer containing a large catalytic subunit (A subunit), encoded in *S. cerevisiae* by two functionally redundant genes, *CNA1* and *CNA2*, and a small regulatory subunit (B subunit), encoded by *CNB1*. Calcineurin pathway interacts with TORC2 and Slm1p and Slm2p and Slm1 proteins are required for TORC2 activation following sphingolipid biosynthesis inhibition (Bultynck et al. 2006). To know whether farnesol mediated increase in AbA resistance involves calcineurin pathway, we analyzed the sensitivity of *cna1Δ* and *cnb1Δ* mutants against AbA, in presence of farnesol. Deletion of *CNA1* or *CNB1* did not affect farnesol mediated increase in AbA tolerance, suggesting increase in AbA tolerance in presence of farnesol, may not involve membrane stress (**Fig. 3b**).

Previous study reported that overexpression of *PDR16* gene confers AbA resistance (Katsuki et al. 2018), similarly mutant strains lacking the ABC transporter *PDR5* and *YOR1* were found to exhibit differential resistance to AbA and the role of these genes in AbA tolerance was well established (Khakhina et al. 2015). Similarly in our study, we also found the consistent role of *PDR5* and *YPK1* in AbA drug resistance modulation, *PDR5* deletant enhances and *YPK1* deletant reduces the farnesol mediated AbA tolerance. In conclusion, we have shown that *PDR5* and *YPK1* is an important regulator of AbA drug resistance. These data further suggest that the existence of *PDR5* and *YPK1* is necessary for farnesol mediated AbA resistance modulation. Efforts are underway to elucidate the exact mechanism of how farnesol affects the sphingolipid pathway for modulating AbA drug resistance.

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Authorship Contribution:

Conceived of or designed study/analyzed data/Wrote the paper VKB,

Performed research/Contributed new methods or models: VM

Compliance with Ethical Standards:

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- Informed consent: Yes

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Figure legend

Fig. 1. (a) Overview of sphingolipid biosynthesis pathway and its regulator in yeast and target site for sphingolipid biosynthesis inhibitor. **(b)** Farnesol modulates AbA drug susceptibilities in strains of *S. cerevisiae* and *Candida*.

Fig. 2. Screening *S. cerevisiae* gene deletants involved in **(a)** sphingolipid biosynthetic regulatory genes **(b)** pleiotropic drug response gene involved in membrane transport. Ten-fold serial dilutions of cells were spotted onto synthetic complete agar plates with indicated concentration of AbA and farnesol (50 μ M). Plates were incubated at 30°C for 2 days before being photographed.

Fig. 3. Screening *S. cerevisiae* gene deletants **(a)** ergosterol biosynthesis and **(b)** calcineurin pathway. Confirmation of the role of *YPK1* in AbA tolerance in presence of farnesol. Ten-fold serial dilutions of cells were spotted onto synthetic complete agar plates with indicated concentration of AbA and farnesol (50 μ M). Plates were incubated at 30 °C for 2 days before being photographed.

(a)

AbA (μg/ml)

(b)

AbA ($\mu\text{g/ml}$)

(a)

AbA ($\mu\text{g/ml}$)

(b)